TWENTY MONTHS OF STARVATION IN AMIA CALVA.[1]

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Early in October, 1911, the department of zoölogy received for class work some forty live *Amia* from Alexander Nielson, Venice, Ohio. At the conclusion of the course, there were six live *Amia* that had not been used. These were left in the basement aquarium room in a zinc tank into which a small stream of the city water was allowed to flow continuously. The fish received no attention.

When college opened in the fall of 1911, the six fish were all alive. During a warm spell in the fall two of them died. It was thought wise to kill and fix the tissues of one of the remaining four for study. This was done. In about a month, a third one died. After this a careful watch was made to note the vigor of the remaining two. In January, 1912, one of the remaining *Amia* was killed and the tissues fixed for study. I was curious to know how long the one remaining fish would live. The individual was a female and she continued to live week after week until June 4, twenty months after being placed in the tank. At this time, the fish had become so emaciated and weak that the long tail would not stand upright and the fish swam feebly. It seemed unwise to carry on the experiment longer for fear of losing the opportunity of fixing the tissues for study. So far as the writer is aware, this is the longest period that a vertebrate has been without food while under direct observation.

The first question to be answered is the organic content of the water. Fortunately during this same period the department of chemistry[2] was making frequent analyses of this same water

[1] Contributions from the Zoölogical Laboratory of Syracuse University, C. W. Hargitt, director.

[2] The following analysis of the city water is approximately correct for the period during which you were working with *Amia calva*. Of course, the chemical composition of any water varies not only from year to year but also from month to month, so that the analysis given, while substantially correct, is not absolutely so. The results are stated in parts per million.

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and a graduate student in bacteriology was making a study of the microörganisms. The latter worked in the same building and took his samples from the laboratory faucets. These two studies were carried on independently and without any reference to mine.

From the chemical analysis of the water one readily observes that the organic content is very low and that there is not enough of the organic compounds dissolved in the water to support such a large fish as *Amia*. The bacteriological study revealed the presence of several species of bacteria of which *B. coli* was the most numerous. The number of protozoa found were very few. Subsequent studies made by the city bacteriological laboratory and extending over a longer period are in general terms as follows: the bacterial content of the city water is from 20 to 40 per cubic centimeter of water when grown on agar at 37° C. After a heavy rain or a quick thaw, the bacterial content is slightly higher.

During part of the time, the water was strained through a fine piece of silk. At the end of two weeks, the silk was removed and the yellowish sediment examined. It was found to consist of diatomes.

These several independent studies show that the organic content of the water is low both in the dissolved organic content as well as in the microörganisms. The next question to be discussed is: Can *Amia* take advantage of this organic material and use any of it as food?

The several writers upon the habits of *Amia*[1] all agree that this fish is a menace to other fish, that it is savage and voracious, eating small fish and crayfish. In its natural habitat, there can

Chlorine	2
Nitrogen in nitrites	0
Nitrogen in nitrates	0.22
Ammonia free	0.04
Ammonia albuminoid	0.015

—Ernest N. Pattee, Director Department Chemistry.

[1] Bean, B., 1896, "On the Dogfish (*Amia calva*), Its Habits and Breeding," Fourth Annual Report, Comm. Fisheries, Game and Forests of New York, p. 249. Bean, B., 1903, "Fishes of New York," p. 75. Jordan and Evermann, "Fishes of North America," Vol. I., p. 113. Reighard, Jacob, 1903, "The Natural History of *Amia calva*," Mark memorial volume, p. 65.

be no question but that it lives upon the large aquatic animals. The effects of this habit is that the gill-rakers are short, blunt processes and between each there is a short space. This means that they are not effective straining organs for minute particles of food. Bacteria and diatomes easily pass between them and out through the gills into the surrounding water. The structure of the gills alone is sufficient to answer the question whether or not *Amia* used the microörganisms as food. I believe that the amount of food secured by these fish from the water is negligible.

The question which Putter,[1] Moore and others have raised in regard to the rôle that organic compounds (other than those ingested) play in nourishing animals receives a negative answer in the case of *Amia* in so far as these experiments are related to the utilization of organic compounds in solution in the water.

Color Changes.

As the breeding season approached, the green color of this female took on a brighter tint that was in sharp contrast to the usual dull color. By the middle of April, this intensifying of the color reached its height and gradually declined during the next three weeks until the regular dull shades were again assumed. On the second return of spring, a similar color change was indulged in. This was surprising as I was accustomed to think of these secondary sexual changes as following a vigorous, well-fed condition. Here the reverse is true as the animal was so emaciated as to be hardly able to swim and then in a very feeble manner. It would seem as if this secondary sexual coloration in *Amia* was a rhythmical process recurring at the period of the breeding season irrespective of bodily vigor.

Blood.

On October 8, 1913, one *Amia* just received from Alexander Nielson was etherized and the blood immediately studied. The

[1] Putter, A., 1907, "Die Ernährung der Wassertiere," *Zeit. f. allg. Physiol.*, 7, Hf. 2 and 3, pp. 283–320. 1909, "Die Ernährung der Wassertiere und der Stoffhaushalt der Gewasser," pp. 1–168, Jena, Vergl. G. Fisher. Moore, Benjamin, Edward D. Edie, Edward Whiteley, W. J. Dakin, 1912, "The Nutrition and Metabolism of Marine Animals in Relation to (*a*) Dissolved Organic Matter, and (*b*) Particulate Organic Matter of Sea-water," Biochem. Jour., vol. 6, pp. 255–297.

specific gravity of this fresh blood was 1.04. In two counts of the red corpuscles, the number was 1,680,000 and 1,600,000; while the white corpuscles were 800,000 and 400,000 in the two counts made. Some of this fresh blood was placed in .5 per cent. osmic acid for later study.

Professor Brewer's[1] chemical analysis of samples of this normal blood gave the total nitrogen in 100 grams as 69 per cent. and the total urea-nitrogen 39.5 per cent. This makes the urea-nitrogen 57.2 per cent. of the total nitrogen in the blood. The remaining nitrogen is in the form of amino-acids.

When the *Amia* that had been starved twenty months was killed, a similar study was made, giving the following results: Specific gravity of the starved blood 1.03. Red corpuscle count 400,000. There was no evidence of white corpuscles in the several counts made nor in the preparations stained with Wright's blood stain. Some of this blood was placed in .5 per cent. osmic acid.

The total nitrogen in 100 grams of this starved blood was 30.5 per cent. and the urea-nitrogen 18 per cent. which gives the urea-nitrogen as 59 per cent. of the total nitrogen in the starved blood.

At the same time that the blood of the normal and of the starved animal was being examined as just indicated, a number of cover glass preparations were made and stained with Wright's stain. These and the osmic fixed corpuscles were subsequently studied with the oil immersion lens. It was soon evident that there was no constant difference between the red corpuscles of the normal and starved animals. But to be more certain, microphotographs were made and the negatives projected onto a screen. In this manner each corpuscle became so large that it was readily measured with a millimeter scale. While these measurements were being made, the negatives of the normal and starved blood were in such order that the one making the measurements did not know whether the blood was normal or starved. When these results were checked up, it was found that the size of the red corpuscles had remained fairly constant. No evidence of any definite variation in the red corpuscles of the

[1] The chemical analyses embodied in this paper were made by Professor R. K. Brewer, M.D., of the Department of Chemistry, Syracuse University.

starved blood was noted. The corpuscles in the blood of the normal fish tended to vary slightly more than those from the starved animal.

Muscles.

The muscles of a normal *Amia* are compact coarse fibers separated by strong connective tissue into myomeres. This muscle layer is from a half to three quarters of an inch in thickness in the dorsal region. When the skin of the starved *Amia* was removed all of the firmness and compactness of the normal muscle was lacking; this was especially true in the apparent disappearance of the myomeres. The muscles in the region of the gills and operculum were similar to the muscles in a normal animal.

When the blade of a scalpel was lightly scraped over the broken down muscles, a murky, structureless substance was secured that flowed from the scalpel in drops when the scalpel was held suspended. A considerable quantity of this semi-fluid muscle tissue was fixed in osmium-bichromate, zenkers, formalin, and chrome-sublimate. One chance preparation was made just as one makes a cover glass preparation of blood and stained with Wright's blood stain. This was fortunate as it was the only one of the preparations to yield satisfactory results for microscopic study.

In preparing for the chemical analysis of this muscle, it was necessary to take all of this semi-fluid muscle tissue in the entire animal in order to secure 3 grams of dry substance.

The following data enables one to compare the composition of the muscle of the normal and starved animals. No fat was found in the starved muscles. For the significance of the following analysis, the reader should consult the numerous papers of Folin[1] and Dennis, and Van Slyke.

[1] Folin and Denis, "Protein Metabolism from the Standpoint of Blood and Tissue Analysis," Seven papers in the *Jour. Biochemistry* as follows: Vol. XI., no. 1, 1912, no. 2, 1912, Vol. XII., no. 1, 1912, no. 2, 1912, no. 2, 1912, Vol. XIV., no. 1, 1913, Vol. XVII, No. 4, 1914. "Metabolism Studies on Cold Blooded Animals," Vol. XIII., no. 2, 1912. "Note on the Tolerance Shown by Elasmobranch Fish Toward Certain Nephrotoxic Agents," Vol. XVI., no. 3, 1913. J. Bio. Chem. Vol. X. P. 15.

The total nitrogen in 3 grams of starved dry muscle was .4474.

	Per Cent.
Ammonia-nitrogen	3.1
Melanin-nitrogen	1.8
Amino-nitrogen	63.9
Non-amino-nitrogen	4.9
Nitrogen of bases	26.9
	100.6

The proportions in the normal muscle are as follows: Total nitrogen in 3 grams of dry muscle .3403.

	Per Cent.
Ammonia-nitrogen	7.2
Melanin-nitrogen	1.9
Amino-nitrogen	55.6
Non-amino nitrogen	9.4
Nitrogen[1] of bases	26.0
	100.1

A comparison of this analysis with that of the starved muscle reveals the interesting fact that the general relation of the several nitrogen compounds found in the muscle is not materially changed. A chemical analysis, therefore, does not help us in explaining the sequence of events which results in the breaking down of the muscle cell.

The histological study of the muscle shows the order in which the parts of the cells disappear, although no satisfactory preparations were obtained from material fixed in the several solutions mentioned above. The semi-fluid of starved muscle stained with Wright's blood stain did however give a fine differentiation of the muscle fibers and their cross striæ.

It has been known for some time that the sarcoplasm was broken down in extreme starvation, but I am not familiar with any observations that determine the order in which the change occurs. The untouched microphotographs, Fig. 1, shows that the cross markings in the sarcoplasm are the first structures to undergo any change. These become faint and less compact at the end of the muscle cell while toward the middle of this same cell they are unchanged. This is clearly indicated in the above figure where a normal fiber and one that is breaking down lie side by side. The muscle nuclei of each fiber are of equal size and staining reaction.

[1] This is the nitrogen precipitated by phosphotungstic acid.

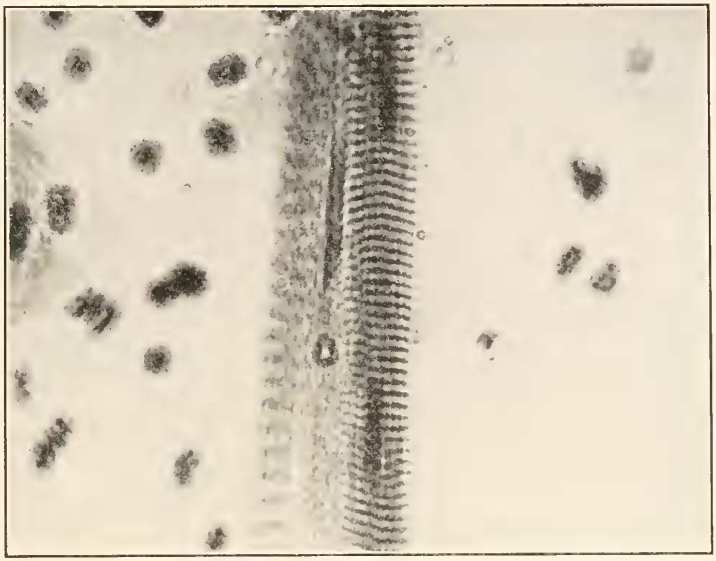

FIG. 1. Microphotograph of body muscle of *Amia calva*, showing normal and partly broken down muscle cells. Stained with Wright's blood stain. Magnification 600 ×.

In Fig. 2, a second microphotograph, are shown some normal muscle fibers, others partly broken down and one entirely empty. In the empty fiber, the muscle nuclei are still arranged along the cell wall of the muscle. One of these nuclei has divided. Three red blood corpuscles appear near this divided nucleus and furnish a good comparison. The appearance of the blood corpuscles as photographed indicates that the cells are well fixed.

These two microphotographs clearly indicate that the striæ, then the sarcoplasm and finally the nuclei is the order in which the several parts of the muscle cell break down in *Amia* during starvation.

Fig. 3 is a microphotograph of a dividing muscle nucleus and the method is certainly amitotical. These nuclei become separated from the cell wall and gradually fragment. Several smaller pieces are seen in this figure.

A variety of stains was tried on the material fixed and sectioned but the results were unsatisfactory. The muscle sarcoplasm and nuclei stained very faintly. In one slide stained with

Conklin's picro-hæmatoxylin many small cells were stained. Each of these has a distinct but small amount of cytoplasm with a definite nucleus in which the chromatin was delicately distributed. These nuclei had a decidedly healthy appearance. After trying a number of stains, I am inclined to interpret them as connective tissue cells. But I am at a loss to understand why

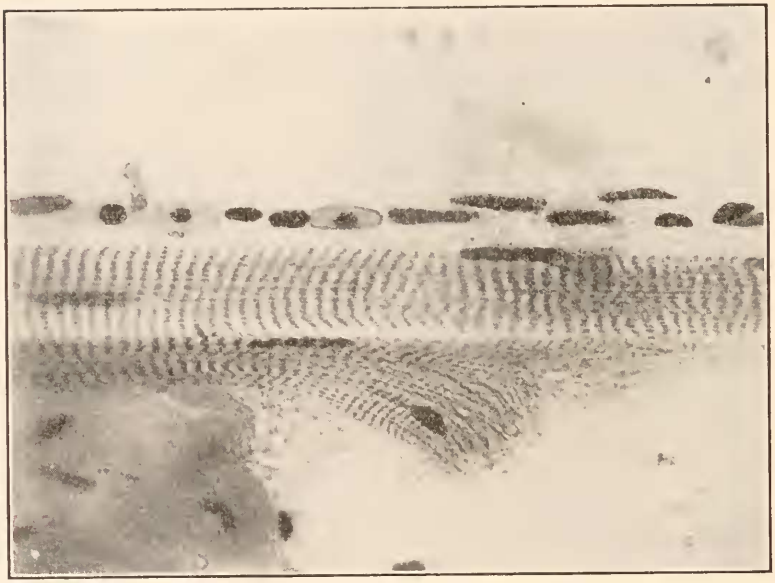

FIG. 2. Microphotograph of body muscle of *Amia calva*, showing a normal partly broken down and an empty muscle cell. Note that the nuclei of the empty cell are still arranged along the cell wall. One has divided. Magnification 600 ×.

they should be apparently so normal when all of the parts of the muscle cell stained so faintly. From their appearance one might suspect that they were associated with the breaking down of the muscle cells. I have not been able to locate in the literature any evidence that the internal secretions that are believed to be responsible for this breaking down of muscle are the product of any definite cells. It may be that part at least of the secret is discovered in these active connective tissue cells.

NERVOUS.

During this prolonged enforced fasting, the operculum was constantly raised and lowered in a regular manner. This simple

movement associated with the passage of water over the gills is correlated with the drawing in of the water into the mouth so that not only the vagus group of nerves but the trigeminal complex also is involved in this apparently simple reflex.[1] In attempting to determine what group of cells was constantly at work in this respiratory movement, one is unable to be certain which group is doing the work. There does not seem to be any

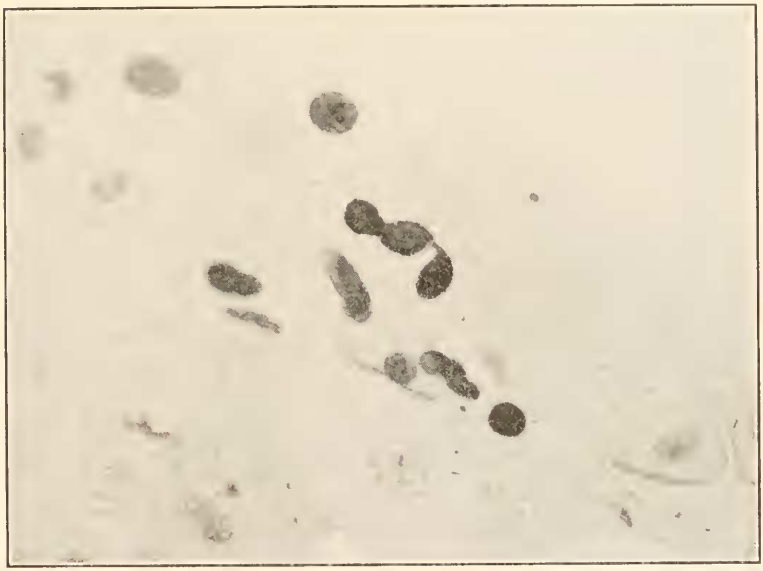

FIG. 3. Microphotograph of amitotically dividing muscle nucleus. The smaller black bodies are muscle nuclei undergoing degeneration. The red blood corpuscles serve as a measure of the amount of change that some of them have undergone.

way of determining which group of cells in the reflex chain is expending the greater amount of energy; is it the group of cells that receives the initiating stimulus or the one that sends the motor stimulus to the muscles of the gills and operculum? Several of the nerve centers associated with the trigeminal and vagus were studied in an attempt to determine the influence of

[1] Allis, E. P., 1897, "The Cranial Muscles and Cranial and First Spinal Nerves in *Amia calva*," *Jour. Morph.*, Vol. XII., no. 3. Herrick, C. Judson, 1899, "Cranial and First Spinal Nerves of *Menidia*, a Contribution upon the Nerve Components of the Bony Fishes," *Jour. Neur.*, Vol. IX.

this continued respiratory activity in the gill region but the results were not satisfactory.

The tank in which these fish were kept was in a shady part of the room and for weeks at a time the fish were not disturbed. It would seem as if the sensory components of these two nerves were as free from stimuli as it is possible to have a living animal in its normal environment. Under such conditions, the cell

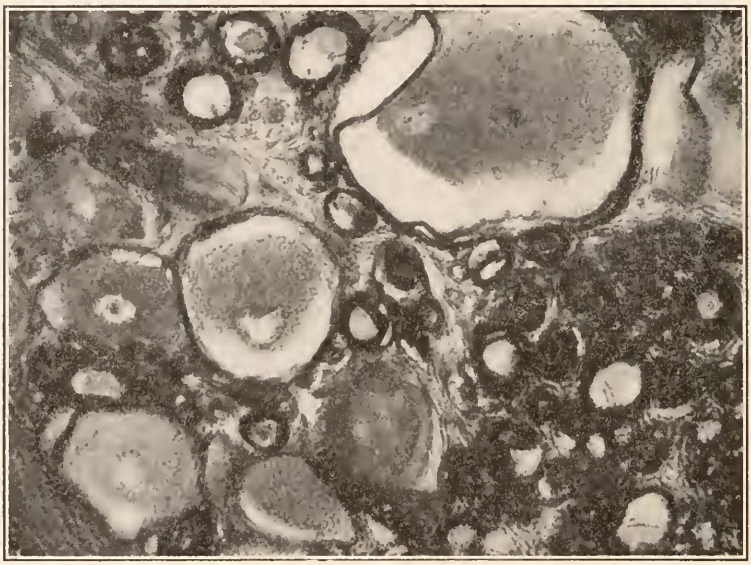

FIG. 4. Microphotograph of nerve cells in tenth ganglion. Magnification 258 ×.

bodies in the tenth ganglion would certainly not be in a state of fatigue. Rather the reverse should be the condition, *i. e.*, a condition of rest. The nerve cells which immediately govern the contraction of the muscles of the gills are located near the floor and at one side of the medulla. In selecting these as the motor nerves of the vagus for the gills, I am accepting Herrick's conclusions as already cited. If these are the correct cells to select for this study, then we should expect them to be in just the opposite condition to the sensory cells in the vagus ganglion because they have been continuously transmitting motor stimuli.

The microphotographs, Figs. 4 and 5, clearly indicate that the

nuclei are round and that the chromatin granules are uniformly scattered. A conspicuous nucleolus is present in nearly every one. The size and general appearance of these nuclei lead me to conclude that the condition of the cytoplasm is also normally fixed. The presence of large clear areas filled with sap is what is found when the living starved nerve cell is studied. It is interesting to note that both of these cells were able to do their normal

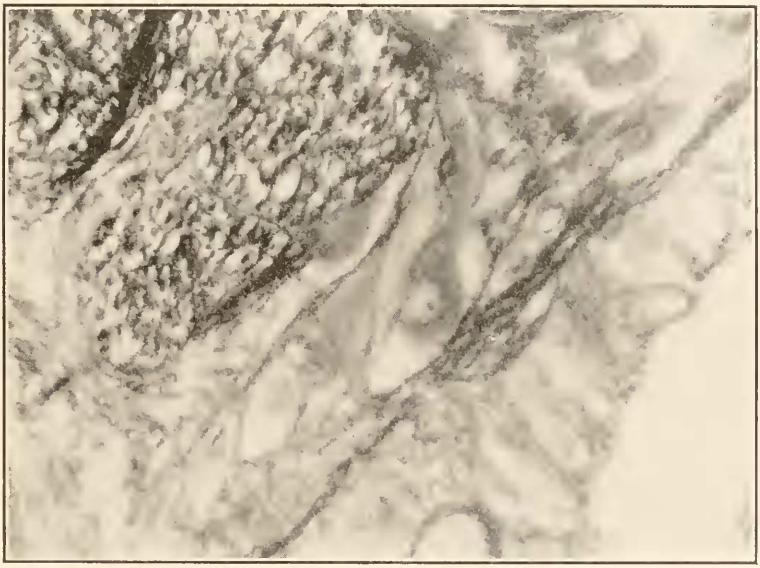

FIG. 5. Microphotograph of motor nerve cells of the tenth nerve. Magnification 258 ×.

work although in an apparent state of almost complete inanition. Many of the cells showed only about one-half of the normal amount of cytoplasm. It is also evident from these two microphotographs that there is no constant morphological difference between the sensory cells that had had a long rest and the motor cells that had been constantly working. In fact so far as I can determine there is no constant structural difference between any of the nerve centers associated with the respiratory reflexes. The fish utilized nearly its entire body muscles in order to supply food energy to the nervous system. This energy while not entirely adequate appears to have been generally distributed

in and utilized by the nervous system irrespective of the amount of work to be performed.[1]

Summary.

1. *Amia calva* is able to live at least for twenty months in an aquarium tank without food. During this time the body was furnished with food energy that was derived from the body muscles.

2. The blood does not show any definite chemical variation during this period of fasting nor do the individual red blood corpuscles undergo a definite change. There seems to be a marked reduction in the number of red and white corpuscles.

3. In the breaking down of the muscle cell, the parts of the cell disappear in the following order: the muscle striæ, then the sarcoplasm and finally the nucleus.

4. The cells of the nervous system continued to function although highly vacuolated. There does not appear to be any constant morphological change in the nerve cells that worked and the cells that rested during this long period of fasting.

5. The bright colors of the reproductive period were assumed by this starved *Amia* twice while undergoing enforced fasting.

[1] A detailed study of the digestive glands and digestive tract is being made by W. H. Kortright and will be reported at a later date.